SUMMARY

Metabolic regulation was studied in the later stage (i.e., in the catabolic stage or in the cachectic stage) of AH-130 ascites tumor-bearing rats. For this purpose, liver L-alanine:2-oxoglutarate aminotransferase activity, liver L-tyrosine:2-oxoglutarate aminotransferase activity, and adrenocortical hormone metabolism were investigated sequentially after tumor transplantation. With regard to the adrenocortical hormone metabolism, adrenal weight, and corticosterone content, plasma corticosterone level and liver $\Delta^4$-3-ketosteroid hydorgenase activity were estimated.

The results showed that the rise in the liver aminotransferase activity of the tumor-bearing rats was associated with an elevated plasma corticosterone level, which was ascribed to the increased hormone production, but not to the suppression of the activity of liver $\Delta^4$-3-ketosteroid hydorgenase, a rate-limiting enzyme in the inactivation processes of the hormone. It was proposed that the increased activity of the liver aminotransferases resulted in the wasting of the host animal through the conversion of body protein to carbohydrate and that cancer cachexia was partly a manifestation of such alterations of the metabolism in the tumor-bearing host. Further, studies were performed with nutritional conditions of high- and low-protein diets and fasting. The results were contrasted to the findings in the tumor-bearing animals to show the specificity of the metabolic regulation in the tumor-bearing animal.

INTRODUCTION

Our previous report (7) showed that the liver tyrosine aminotransferase activity was increased in the later stage in tumor-bearing animals and that such increment of the activity was abolished by adrenalectomy. It is now well established that the liver aminotransferase activity is increased after administration of adrenocortical hormone and that the increased activity of this enzyme facilitates the use of amino acids or body protein as the energy source (9, 16, 17). The possibility that the increased activity of the liver aminotransferase in the tumor-bearing animal, an adaptive response of the host animal, might be mediated by an altered metabolism of adrenocortical hormone was also suggested (7). However, there were some discrepancies among the results reported on the adrenocortical hormone metabolism in tumor-bearing animals (6, 10, 11, 13, 14, 18, 19).

In the present study, with AH-130 ascites tumor-bearing rats, the activities of alanine and tyrosine aminotransferases and of catalase were measured in the liver as indices of the metabolic abnormalities of the tumor-bearing rats. With regard to the function of the adrenals, adrenal weight and corticosterone content and plasma corticosterone level were determined. In addition, liver $\Delta^4$-hydrogenase activity was measured. Inactivation rate of adrenocortical hormone is limited by this enzyme (24). The significance of the findings were discussed in relation to the state of cancer cachexia. Moreover, investigations were also made on the metabolic changes under various nutritional conditions which might affect the metabolic state of the tumor-bearing animal.

MATERIALS AND METHODS

Animals and Diets. Male Wistar rats, weighing about 100 g, were used in all experiments. Each group consists of 5 rats unless otherwise indicated. Routinely, they were maintained on a standard diet of Oriental pellets NMF and water ad libitum. Special diets of various protein levels (Table I) were prepared and given to the rats for 7 days in order to investigate the effect of the dietary regulation.

Transplantable Tumor. AH-130 ascites tumor was used. The ascites, 0.3 ml, was inoculated i.p. into each rat. Mean survival time of the tumor-bearing rats was about 3 weeks.

Enzyme Assays. Rat liver was homogenized for 2 min in a Potter-Elvehjem homogenizer with a Teflon pestle. The liver homogenate thus prepared was used as such for the enzyme assay. The unit of activity of the enzymes was expressed as 3

1This work was supported in part by a grant from the Ministry of Education.
2Present address: 4-1-1, Sannomaru, Naka-ku, Nagoya, Japan.
Received June 5, 1969; accepted September 24, 1969.

1The abbreviations used are: alanine aminotransferase, L-alanine:2-oxoglutarate aminotransferase (EC 2.6.1.2); tyrosine aminotransferase, L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5); catalase, $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$ oxidoreductase (EC 1.11.1.6); $\Delta^4$-hydrogenase, $\Delta^4$-3-ketosteroid hydorgenase.

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AH-l 30 ascites tumor was inoculated intraperitoneally into rats in volume of 0.3 ml. Mean survival time of the tumor-bearing rats was about 3 weeks.

Corticosterone was measured principally by the method of Tomkins (23) with corticosterone as substrate at pH 7.4. Corticosterone is reduced by this enzyme to form the physiologically inactive compound dihydrocorticosterone.

Catalase. The condition of assay (21) was based on the method of von Euler and Josephson (22).

$\Delta^4$-Hydrogenase. In rats, secretion of corticosterone is predominant (3). For the estimation of this enzyme activity, the method of Tomkins (23) was used, with corticosterone as substrate at pH 7.4. Corticosterone is reduced by this enzyme to form the physiologically inactive compound dihydrocorticosterone.

Corticosterone. Both adrenal and plasma corticosterone levels were determined by the method of Silber et al. (12). The fluorescence of corticosterone was read in a Hitachi Model A-2 fluorospectrophotometer.

### Table 1
Composition of experimental diets

<table>
<thead>
<tr>
<th>Composition</th>
<th>Low-protein diet, (g)</th>
<th>Control diet, (g)</th>
<th>High-protein diet, (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(5% casein)</td>
<td>(20% casein)</td>
<td>(60% casein)</td>
</tr>
<tr>
<td>Casein</td>
<td>5</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>Corn starch</td>
<td>65</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>Oil a</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Salt mixture b</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixture a</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

a Tanabe Amino Acids Research Foundation.

### RESULTS

#### Tumor-bearing State

Table 2 shows the changes in liver alanine aminotransferase and tyrosine aminotransferase activities after tumor transplantation. No significant changes occurred in the activities of these enzymes at Days 1, 3, 5, 7, and 9. On the 14th day after tumor transplantation, when the ascites volume reached 50 ml, both tyrosine aminotransferase and alanine aminotransferase activities were significantly elevated above the control, the increases being 2-fold and 1.8 times, respectively. Thus, the activity of the 2 aminotransferases increased in the advanced stage of tumor growth.

Table 2 also shows the sequential alterations of plasma corticosterone levels after tumor transplantation. A significant elevation was observed only on the 14th day of transplantation, the value being 70 to 80% higher in plasma of tumor-bearing animals compared to plasma of control animals. Corticosterone metabolism was investigated by studying $\Delta^4$-hydrogenase activity, an enzyme which degrades corticosterone. The activity of this enzyme in liver was unchanged throughout the period studied. Thus, elevation in plasma corticosterone was not due to a decrease in its metabolic degradation.

An attempt was made to correlate the change in the adrenal weight and its corticosterone content to the hormone-generating ability of the adrenal gland. As shown in Table 2, no significant changes were observed in weight and corticosterone content of the adrenals of tumor-bearing animals at Days 1, 3, 5, 7, and 9. After 14 days of transplantation, both adrenal weight and corticosterone content were significantly increased, the increases being 60% higher in both parameters of tumor-bearing animals compared to those of control animals. The corticosterone concentration, as calculated on the basis of the wet weight of adrenals, did not differ between the control and the tumor-bearing rats throughout the experiment. It might be

### Table 2
Sequential changes in liver aminotransferase activity and corticosterone metabolism after tumor transplantation

<table>
<thead>
<tr>
<th>Days after transplantation</th>
<th>Alanine aminotransferase</th>
<th>Tyrosine aminotransferase</th>
<th>$\Delta^4$-Hydrogenase</th>
<th>Plasma corticosterone level (mM/100 ml)</th>
<th>Adrenal weight (mg/100 g body weight)</th>
<th>Adrenocorticosterone content (mM/100 g body weight)</th>
<th>Adrenocorticosterone/adrenal (mg/mg adrenal weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Tumor-bearing</td>
<td>Control</td>
<td>Tumor-bearing</td>
<td>Control</td>
<td>Tumor-bearing</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>4.740 ± 450</td>
<td>522 ± 36</td>
<td>25.4 ± 7.4</td>
<td>17.8 ± 2.1</td>
<td>16.1 ± 0.8</td>
<td>0.67 ± 0.12</td>
<td>0.042 ± 0.006</td>
</tr>
<tr>
<td>3</td>
<td>4.740 ± 450</td>
<td>4.980 ± 522</td>
<td>24.4 ± 6.2</td>
<td>17.8 ± 2.1</td>
<td>16.1 ± 0.8</td>
<td>0.67 ± 0.12</td>
<td>0.042 ± 0.006</td>
</tr>
<tr>
<td>5</td>
<td>4.320 ± 288</td>
<td>4.290 ± 322</td>
<td>25.4 ± 7.4</td>
<td>21.4 ± 5.2</td>
<td>17.6 ± 1.7</td>
<td>0.56 ± 0.02</td>
<td>0.042 ± 0.006</td>
</tr>
<tr>
<td>7</td>
<td>4.380 ± 246</td>
<td>4.440 ± 330</td>
<td>24.0 ± 6.2</td>
<td>21.4 ± 5.2</td>
<td>17.6 ± 1.7</td>
<td>0.56 ± 0.02</td>
<td>0.042 ± 0.006</td>
</tr>
<tr>
<td>9</td>
<td>594 ± 48</td>
<td>684 ± 120</td>
<td>26.5 ± 7.7</td>
<td>23.0 ± 5.2</td>
<td>13.9 ± 1.1</td>
<td>0.63 ± 0.01</td>
<td>0.039 ± 0.003</td>
</tr>
<tr>
<td>14</td>
<td>5.700 ± 582</td>
<td>9.180 ± 780</td>
<td>21.8 ± 1.4</td>
<td>19.9 ± 1.5</td>
<td>15.6 ± 1.0</td>
<td>0.65 ± 0.07</td>
<td>1.02 ± 0.12</td>
</tr>
</tbody>
</table>

a The unit of activity of the enzymes was expressed as $\mu$moles product formed per hr on a 100-g body weight or carcass weight (body weight minus ascites) basis. Each value represents the mean ± S.E. of 5 rats, except the 14th-day values, which represent the results from 10 rats in each group.

bSignificantly different from the mean of the control at a p value of 0.01 or less.

cSignificantly different from the mean of the control at a p value of 0.05 or less.
possible that the enlargement of the adrenals resulted in the hyperfunction of the adrenals which in turn reflected in the plasma corticosterone level of the tumor-bearing rat.

**Comparison of Tumor-bearing State with That of Various Nutritional Regulations**

It is assumed that the metabolic abnormalities in tumor-bearing animals is related to a state of nutritional disturbance. For clarification of this problem, studies were made on the nutritional influences on the metabolism of tumor-free rats. The nutritional conditions selected were 3-day fasting, high-protein diet, and low-protein diet. Casein was used as protein source of the diets. The composition of each diet was listed in Table 1. Body weight changes under these nutritional conditions were shown in Chart 1. A marked suppression of weight gain in the group fed low-protein diet and a significant loss of weight in the 3-day-fasted group were noted.

**Three-Day Fasting.** Results are shown in Table 3. An increased activity of the liver aminotransferases and an elevated plasma corticosterone level were also observed as in the tumor-bearing rat. On the other hand, the liver \( \Delta^4 \) -hydrogenase activity decreased in the 3-day-fasted group. It was thought that, in contrast to the tumor-bearing group, suppression of the inactivation process of the adrenocortical hormone played an important role for the elevation of the plasma corticosterone level in the fasted group.

**High-Protein Diet.** As shown in Table 3, the activity of liver aminotransferases was also increased on the high-protein diet. No changes occurred in plasma and adrenocorticosterone content. It was also observed that the liver \( \Delta^4 \) -hydrogenase activity was not changed with the high-protein diet. Further, the high-protein diet did not affect the liver catalase activity (Chart 2), whereas a significant decrease in the liver catalase activity was observed in the tumor-bearing animals (4).

**Low-Protein Diet.** The activity of alanine aminotransferase, \( \Delta^4 \) -hydrogenase, and catalase in the liver decreased on the low-protein diet (Chart 2). No significant changes occurred in adrenal weight and plasma and adrenocorticosterone contents (Table 3).

Thus, it was concluded that the metabolic abnormalities observed in tumor-bearing rats were to be distinguished from the state of nutritional shift which was produced by various dietary conditionings.

**DISCUSSION**

The present paper deals with the comparative study on the regulatory mechanism of the metabolism between the tumor-bearing and normal animals, with special references to the liver aminotransferase activity and the adrenocortical hormone metabolism.

The increased activity of the enzymes of amino acid catabolism, liver alanine and tyrosine aminotransferase, was demonstrated in the later stage of the tumor-bearing animal, and the abolishment of such increment of the enzymes was

![Chart 1. Body weight changes under various dietary protein levels and fasting. Each point represents the mean of 5 rats.](chart)

---

**Table 3**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Alanine aminotransferase</th>
<th>Tyrosine aminotransferase</th>
<th>( \Delta^4 ) -Hydrogenase</th>
<th>Plasma corticosterone level (( \mu g/100 ) ml)</th>
<th>Adrenal weight (mg/100 g body weight)</th>
<th>Adrenocorticosterone content (( \mu g/100 ) g body weight)</th>
<th>Adrenocorticosterone/adrenal (( \mu g/mg ) adrenal weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% casein (control)</td>
<td>3,890 ± 165</td>
<td>588 ± 109</td>
<td>30.4 ± 3.7</td>
<td>17.1 ± 1.0</td>
<td>18.3 ± 1.1</td>
<td>0.70 ± 0.08</td>
<td>0.037 ± 0.006</td>
</tr>
<tr>
<td>3-day-fasted</td>
<td>9,718 ± 8.20b</td>
<td>1,160 ± 180c</td>
<td>18.0 ± 3.7</td>
<td>29.6 ± 5.1c</td>
<td>21.5 ± 0.9</td>
<td>0.79 ± 0.05</td>
<td>0.038 ± 0.005</td>
</tr>
<tr>
<td>5% casein</td>
<td>1,828 ± 2.84b</td>
<td>412 ± 48</td>
<td>10.0 ± 1.9b</td>
<td>22.7 ± 2.5</td>
<td>17.6 ± 0.6</td>
<td>0.69 ± 0.05</td>
<td>0.039 ± 0.002</td>
</tr>
<tr>
<td>60% casein</td>
<td>12,186 ± 1.030b</td>
<td>2,190 ± 192</td>
<td>29.4 ± 5.4</td>
<td>23.1 ± 3.4</td>
<td>22.5 ± 1.2c</td>
<td>1.00 ± 0.14</td>
<td>0.045 ± 0.006</td>
</tr>
</tbody>
</table>

\( a \) The unit of activity of the enzymes was expressed as \( \mu \) moles product formed per hr on a 100-g body weight basis. Each value represents the mean ± S.E. of 5 rats.

\( b \) Significantly different from the mean of the control at a \( p \) value of 0.01 or less.

\( c \) Significantly different from the mean of the control at a \( p \) value of 0.05 or less.
S. Suga, H. Mekata, T. Mizuno, and Y. Kato

observed by either adrenalectomy or glucose administration (7). It was reported (7) that no particular change was observed in the activity of glucocorticoid-independent enzyme, liver L-tyrosine:pyruvate aminotransferase, in the later stage of the tumor-bearing state. These observations support the view that adrenocortical function is involved in the increased activity of the liver aminotransferase in the catabolic stage of the tumor-bearing animal. Wood et al. (25) also suggested the presence of the hyperfunction of the adrenals in tumor-bearing mice with regard to the changes in the liver tryptophan peroxidase activity. As for the adrenocortical hormone metabolism, there is evidence which indicates that the biosynthesis of the hormone increases in the tumor-bearing animal, i.e., an increase was observed in the adrenal weight (1, 6, 9), in the plasma corticosteroid level (6, 14), and in the urinary corticosteroids (13, 14, 19). On the contrary, Shiba et al. (18) reported that plasma corticosterone level decreased in tumor-bearing rats. The present experiments showed that a high plasma corticosterone level is associated with the increase in weight and corticosterone content of the adrenals of the tumor-bearing rats. With regard to the inactivation processes of the adrenocortical hormone, King and Gordon (10) reported that liver Δ4-hydrogenase activity was increased in the course of tumor growth. On the contrary, a decreased activity of this enzyme was reported by Konishi et al. (11) at the later stage of tumor growth. The liver Δ4-hydrogenase activity determined in this experiment showed no particular change as compared with that of the control throughout the whole experimental period. To sum up the various results mentioned above, the presence of adrenocortical hyperfunction was indicated in the tumor-bearing animals, and the activity of liver aminotransferases was increased by the hormone.

The investigation of the nutritional conditions such as the intake of diet with high or low protein content or the fasting may provide us with valuable information for better understanding of the tumor-bearing state. The increment of tyrosine aminotransferase activity (16) and the hyperfunction of the adrenals (2, 20) were observed in the fasting rats. In contrast, liver Δ4-hydrogenase activity was decreased by fasting (5). In this respect, the fasting differed from the tumor-bearing state where the liver Δ4-hydrogenase activity remained unchanged. Rosen et al. (16, 17) observed that the liver alanine and tyrosine aminotransferase activities were increased significantly with a high-protein diet. In the present experiment, it was demonstrated that the activity of alanine and tyrosine aminotransferases was increased with the high-protein diet, without any changes in plasma and adrenocorticosterone contents. The liver Δ4-hydrogenase activity was not changed. It is likely that the high-protein diet produced a substrate induction of the aminotransferases. Further, the high-protein diet did not cause a decrease of liver catalase activity, which is the most common phenomenon in the tumor-bearing animal (4). The activity of liver alanine aminotransferase decreased on the low-protein diet. It was also observed that the liver Δ4-hydrogenase activity decreased on the low-protein diet without significant changes in plasma and adrenocorticosterone contents. No definite conclusion may be reached with these findings at this time.

Thus the metabolism in the tumor-bearing animal, especially during the later catabolic or cachectic stage, showed some traits which are specific for the tumor-bearing animal. For better understanding of such specificity of the metabolism in the tumor-bearing animal, the participation of humoral factors elaborated by the cancer tissue will have to be taken into consideration.

ACKNOWLEDGMENTS

We are greatly indebted to Dr. Susumu Hibino, Ex-Professor of the First Department of Internal Medicine, Nagoya University School of Medicine and President of National Nagoya Hospital, for his encouragement and advice.

REFERENCES


Chart 2. Liver catalase activity under various dietary protein levels and fasting.
Aminotransferase and Corticosteroid Metabolism


Relationship between Liver Aminotransferase Activity and Adrenal Cortical Hormone Metabolism in Tumor-bearing Rats

Shoji Suga, Hidetoshi Mekata, Takahiko Mizuno, et al.


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